Otolith Aging and Analysis

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Abstract

Otoliths, also known as earstones are paired calcified structures used for balance and hearing in teleost fish. An otolith is acellular and metabolically inert providing biologists with a record of exposure to both the temperature and composition of the ambient water. Otoliths provide an abundance of information ranging from temperature history, detection of anadromy, determination of migration pathways, stock identification, use as a natural tag, and most importantly age validation. Growth rings (annuli) on the otolith record the age and growth of a fish from birth to death. With the use of Matlab the goal of this project is to design a program that uses digital otolith images to semi-automate the aging process. There are three main components to this task. The first is to clear up the image making each annuli in the otolith distinct. The second is to count the number of annuli from the focus of the otolith to the edge and measure distances between each annuli. The third component is to use different backcalculation models to estimate a fish's length at age n. Results from the image enhancement process greatly increased the contrast, which in turn provided more accurate results for the semi-automatic aging approach. However, this approach to aging decreases in accuarcy as the number of annuli increase. For this reason a manual measurement was added to the program allowing the user to select each annulus simply by clicking on it. For fish with five or less annuli the semi-automatic and manual methods have the same accuracy. The otolith aging outputs for this program are annuli estimate, number of rays, distance from the nucleus to each annulus and the edge, and standard deviation.

Section 1

Introduction

1.1

Aging structures

Fish age and size are two of the most important pieces of biological data found in fisheries research. It is the foundation on which fisheries management is built. With this information biologists can infer about population, growth rates, age specific estimates of stock biomass, mortality rates and predictions of future stock conditions. Biologists can use several different structures within the fish to acquire information regarding fish age. These structures include scales, fin rays, vertebrae, and most importantly otoliths. Scales can be difficult to read due to resorbtion. The benefit of scales, unlike other structures, is that samples can be taken without having to kill the fish. Fin rays to some extent can also be collected without mortality. While otolith collection requires killing the fish, this is the most widely used strucutre. The reason being, they generally are almost always easiest to read. Often times several structures are used to achieve the most accurate aging results.

1.2

Otoliths

Otoliths sometimes called earstones are hard calcified carbonate structures located in the brain casing of all teleost fish. Fish use them for hearing and balance, but biologists use them for aging and growth studies. Otoliths are popular because compared to other structure they generally provide the most accurate ages, mainly due to their continued growth throughout the life of the fish. Otoliths are acellular which, implies that they are not subject to resorption. This gives them an advantage over aging scales. Each fish has three types of otoliths; sagittae astericii, and lapilli. Sagittae are the largest and most commonly used for aging. The sagittae are involved in the detection of sound, converting sound waves into electrical signals. Asteriscii, which are smaller than the sagittae, are also involved in the detection of sound. If accurate ages can not be estimated using the sagittae it is not uncommon to use the asteriscii as backup. Finally the lapilli, usually the smallest of the three pairs of otoliths, are used for the detection of gravitational force and sound. (Popper and Lu 2000). The lapilli are hardly ever used for aging

Otoliths vary in shape and size depending on the species. Researchers who analyze stomach contents occasionally use otolith shape to help identify fish species. In addition, other studies have shown otoliths being used to record temperature history (Patterson et al. 1993), whether a fish is anadromous (Secor 1992), migration pathways (Thresher et. al. 1994), stock identification (Edmonds et. al. 1989), or have been used as a natural tag (Campana et. al. 1995).

1.3

Annuli formation

Otoliths, as well as other boney structures form yearly rings (similar to that of a tree) known as annuli. Each annulus is composed of opaque and translucent zones, which correspond to periods of fast and slow growth (figure 1). As a fish begins its life it lays down daily rings as a result of an internal clock which is entrained by a 24-hour light and dark cycle. In addition environmental factors such as feeding, activity and temperature variations all contribute to the daily cycle (Campana and Neilson, 1985). During periods of slow growth daily rings form extremely close together creating a thick band or annulus. In general, the opaque zone forms during periods of increasing water temperatures, while the translucent zone is formed during periods of reduced growth which may be associated with spawning. The formation of annuli is not always clear cut. Unusual weather may provide a period of increased food resources during otherwise poor conditions. This could result in a temporary period of fast growth which might leave an impression of a false annulus.

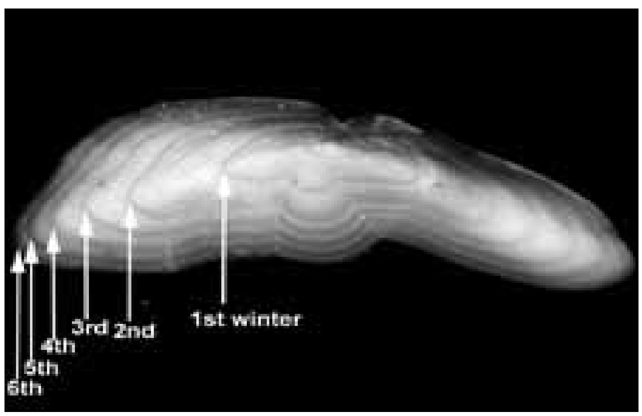


Figure 1.
This was taken from a cod that has six annuli. Each annuli is indicated by arrows. Courtesy of Otolith research laboratory Bedford institute of Oceanography.

1.4

Otolith preparation and interpretation

Otolith preparation is a very time consuming process. It requires a microscope slide, some type of thermoplastic glue, and a means to section the otolith either by grinding with sand paper or a diamond blade wet saw. The idea is to grind the otolith from either side until a thin section containing the nucleus remains. Depending on a person's experience this can take up to an hour. Once this is completed the sectioned otolith is mounted onto a slide then read by an experienced technician.

Upon completion of otolith interpretation a reader must then measure the distance between annuli in order to perform a backcalculation. Backcalculations estimate the fish's length at a previous age. The reader determines the ratio between otolith length and fish length. For example if the relationship is linear than the increment width between annuli are proportional to the growth of the fish. There are several models used to describe this relationship. One of the traditional models is the Fraser-Lee, which is similar to a linear regression model. However, most species otolith:fish length relationships are not exactly linear. A number of studies have shown that otoliths of slow growing fish are generally larger and heavier than those of fast growing fish of the same size. To account for the difference in growth rate, the biological intercept model was created. Similar to the Fraser-Lee model, this model assumes a linear relationship, but adds a biological intercept that is determined by the mean size of the fish and otolith at the larval or juvenile stage (Campana and Jones 1992). A third approach is the Weisburg model. This approach uses a linear model to separate age and year specific effect on otolith growth similar to a two-way analysis of variance (Klumb et. al. 2001). The model accounts for environmental influences. When choosing the best model, it is important to understand something about the life history of the species in question.

1.5

Important otolith features

When analyzing images, there are a few important features that must be noted. Annuli are generally oriented parallel to the outer surface of the otolith. They surround the core or nucleus which in most cases is translucent. Near the core is the sulcus which is a longitudinal groove through which an auditory nerve passes. Many times annuli are most clear near the sulcus (figure 2). Interpretation of otoliths takes a lot of practice. Depending on the experience of the technician there are a wide variety of problems associated with preparation. Improperly prepared otoliths can result in the absence of annuli. In many cases abnormal periods of rapid growth caused by unusual environmental conditions can create splits in annuli or even perhaps a false annulus. The first annulus in figure 2 shows an example of a split.

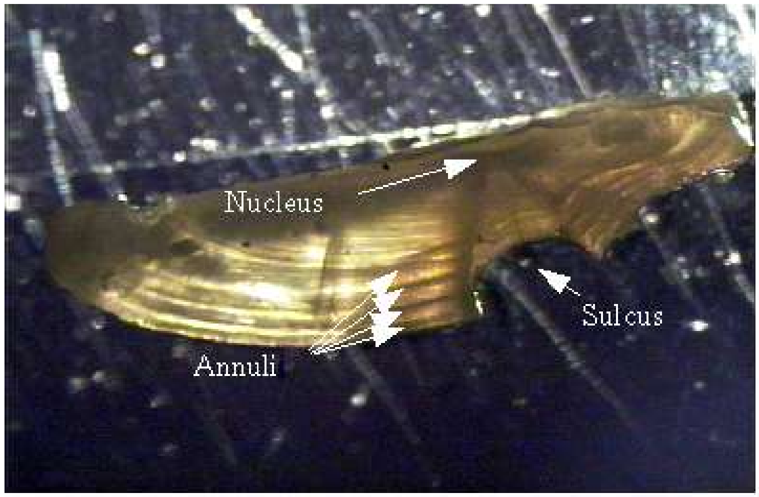


Figure 2.
Three important structures to note when digitally interpreting images. The nucleus, annuli, and sulcus. Also note a split in the first annulus near the sulcus.

1.6

Objective

Currently, the research on digital otolith aging is minimal. A few studies have attempted to estimate age using statistical learning methods (Fablet et. al. 2004). The objective of this project is to use some original and current methods to develop a user friendly program in Matlab® that analyzes, enhances and interprets digital images of otoliths based on intensity profiles. In addition this program calculates otolith:fish length ratios to be applied to a backcalculation model.

Section 2

Materials and Methods

2.1

Image capturing

Images are from already prepared otoliths of largemouth bass (*Micropterus salmoides*), white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*) collected from Lake Pleasant just north of Phoenix Arizona. A digital camera attached to a light microscope was used to capture images. Xcap, an image capturing software was used to collect the images that were then analyzed using Matlab 7 (R14)®.

2.2

Image enhancement

The first step was to read the image into the program and change the image from a colormap to a grayscale intensity image. A contrast limited adaptive histogram equalization (CLAHE) was the method used to enhance the image. The contrast of the image is enhanced by transforming the intensity values using CLAHE. The image is subdivided into nXm blocks or tiles. The tiles are specified by the user prior to enhancement. Each tile's contrast is enhanced so that the histogram of the output region approximately matches a flat histogram. The minimum and maximum tile range is 2 X 2 and 50 X 50 respectively.

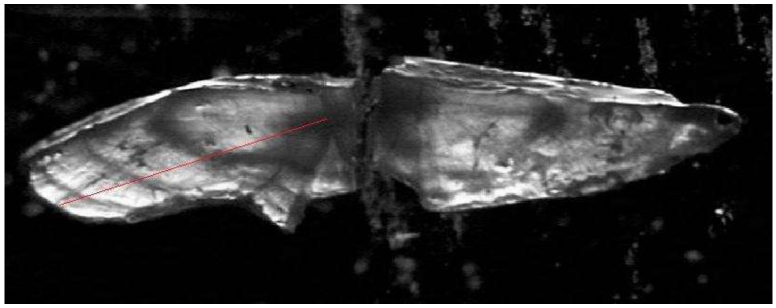
2.3

Counting and measuring annuli

To measure annuli the program is designed to allow the user to select a manual or semi-automatic approach to counting. The impixel function is used for manual measurement. Impixel allows the user to select points on an image returning the coordinates as well as the intensity value of each point. Once all selections are made, the number of points are counted and distances are between points are measured. The distances between each annuli are applied to one of several backcalculations to establish length at age of an individual fish.

The semi-automatic approach evaluates the intensity profile along a specific path chosen by the user from the nucleus to the otolith's edge. The improfile function selects equally spaced points along the path and then uses interpolation to find the intensity value for each point (figure 3a and 3b). The mean of the pixel intensities along this path is calculated. In most cases the pixel intensities of the nucleus are below the mean of the pixel intensities along the specified path. Because this is the case a linear fit is calculated at the point along the profile when the intensity value goes from below the mean to above. This is done to provide a more accurate linear fit of the annuli. At this point the negative residuals from the linear fit are measured because they represent the areas of low intensity caused by the annuli (figure 4). To account for false residuals (residuals that do not represent annuli), a stringency factor is applied. The stringency factor ranges from 0.1 to 0.5 and is selected by the user. The stringency factor is multiplied by the median of the residuals to provide a threshold determining the annuli count. Any residual above this threshold will be counted as an annulus.

a)



b)

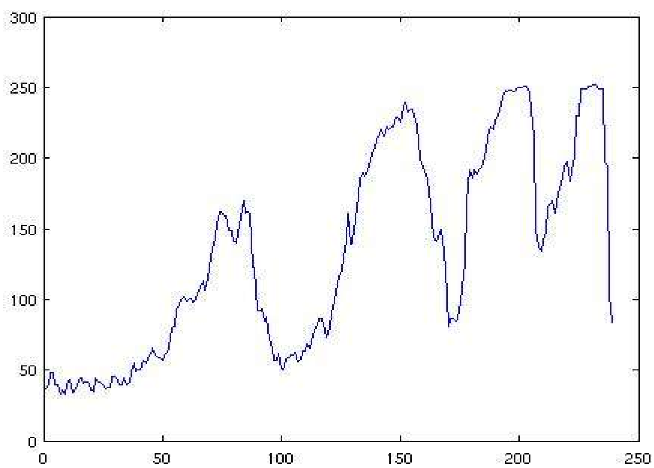


Figure 3.
a) Shows a profile line (red) along the otolith starting from the nucleus and ending at the edge. b) The output of the intensity profile from the profile line. Notice the nucleus x < 50 has low intensity values. There are three annuli seen here by the three valleys around x = 100, x = 175 and x = 210. This is followed by a drop off in intensity at the edge.

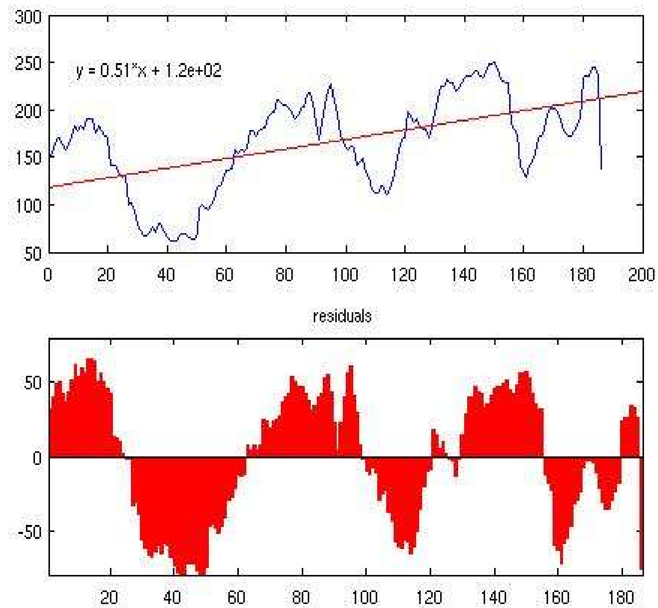
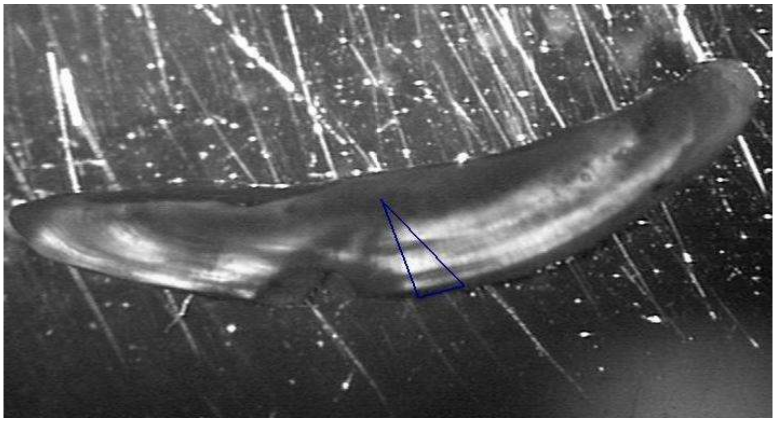


Figure 4.
The top figure shows the linear fit of the plot profile for an otolith that contains three annuli. The lower figure shows the residuals (red) of the linear fit. Note that there are three main negative residuals and one smaller residual between the second and third annuli. This small residual will be dismissed during the counting process.

The next step uses the improfile concept and applies it to a range of area on the otolith selected by the user. A triangular area of interest is selected with the starting point at the nucleus and it's opposite edge along the otolith's border (figure 5a). The distance of the triangle's edge along the otolith border is calculated. Interpolation is used to determine the number of unique pixels along this edge. A series of rays and their intensity profiles are calculated from the nucleus to each unique pixel along the edge (figure 5b). The mean of count of the rays is calculated to determine the final age estimate. The first ray in the series that has the same count as the final estimate is used to calculate distance between annuli..

a)



b)

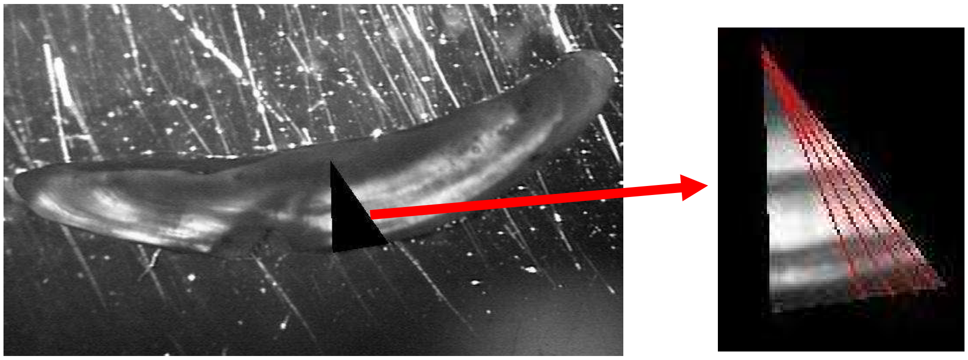


Figure 5.
a) A triangular region of interest (blue) selected by the user. For the best results, the region will generally encompass the clearest area on the image. b) An example of a series of rays and how they are measured. Each ray represents an intensity profile from the nucleus to the outer edge of the otolith.

2.4

Graphical User Interface

The user interface was designed using the GUI guide in Matlab®. The interface is set up in a manner that allows the user to browse for an otolith image. The image appears on the right hand side of the window. A radio button lets the user choose between a manual measurement or a semi-automatic measurement. A slider called enhancement was created allowing the user to select the value of the nXm matrix for the histogram equalization. When the enhancement button is push the image is updated in its enhanced form. Another slider can be adjusted to select the stringency factor. On the lower left hand corner of the screen is the output, which provides the results of the estimation of annuli, number of rays used in the estimation, standard deviation, and the distances between annuli. In addition a fish size text box allows the user to type in the size of the fish for backcalculation purposes. A yellow x is used to mark each annulus and the outer edge. A red x is used to identify the nucleus of the otolith.

Section 3

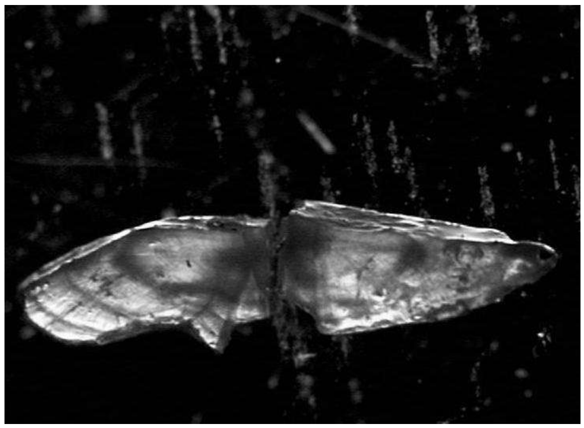
Results

3.1

Image enhancement

The results of image enhancement show a greater contrast when using the adaptive histogram equalization versus no enhancement at all (figure 6a and 6b). Table 1a. and 1b shows the peak and valley differences for each annulus on a three year old largemouth bass. The mean difference for the non-enhanced image is 122 (table 1a.). For the enhancement a 20 by 20 block was chosen for the histogram equalization. Figure 7a and 7b shows the intensity profile for the non enhanced image and the enhanced image respectively. Table 2 shows the peak and valley differences for each annulus of the enhanced image. The mean difference for the enhanced image is 197 (table 1b). The enhancement difference between images is 75. The results also showed the number of residuals increased with image enhancement. The residuals however were factored out when the stringency factor was applied.

a)



b)

Figure 6.
a) A three year old largemouth bass before image enhancement. b) The same image as a) after image enhancement. To enhance this image a 20 by 20 block was used for the image histogram equalization.

a)

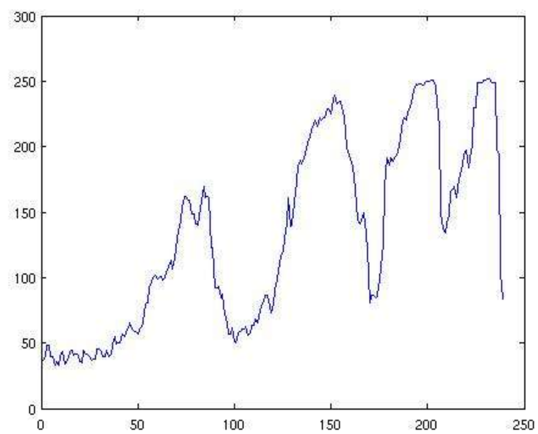
	1st Annulus	*2nd Annulus*	*3rd Annulus*	*Mean*
Peak	160	230	240	
Valley	50	75	140	
Difference	110	155	100	122

b)

	1st Annulus	*2nd Annulus*	*3rd Annulus*	*Mean*
Peak	210	225	250	
Valley	20	45	30	
Difference	190	180	220	197

Table 1.
a) The local min and max for the peaks and valleys representing annuli of the non-enhanced image. b) The local min and max for the peaks and valleys representing annuli of the enhanced image using a 20 by 20 block.

a)



b)

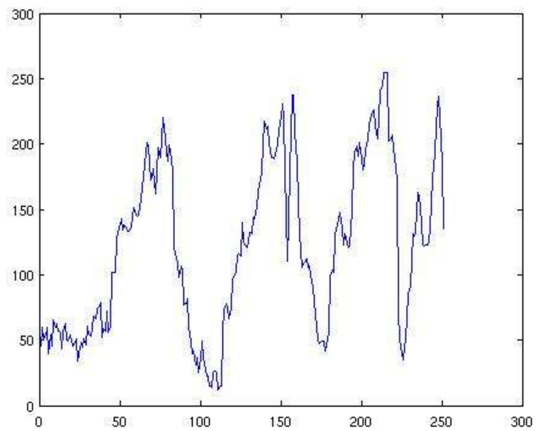


Figure 7.
These profiles come from a three year old largemouth bass. a) The intensity profile of a non-enhanced image. b) The intensity profile of an enhanced image. Notice the increase in noise after enhancement.

3.2

Manual count

The results on a test run for a three year old largemouth bass are as follows. Figure 8 shows the coordinates and intensities of each of the selected points on the image. For most images there is an increase of intensity as you move farther away from the nucleus. The nucleus almost always has a lower intensity value than the annuli. The intensity values from the nucleus to the edge for the image in figure 8 are 33, 62, 118 , 157, and 24. The lower the intensity value the darker the pixel. Table 2 shows an increase in intensity as you move away from the nucleus towards the third annulus. At the edge of the otolith the intensity value drops dramatically. This dramatic drop tells the program where the edge of the otolith is located. So it is important for the background of the image to be dark. The distance to the first annulus is 114, to the second is 189, to the third is 235 and to the edge is 264. In order to enter data into a backcalculation model it is necessary to know the ratios between annuli. The ratios shown in table 3 are the distances from the nucleus to annulus n divided by the distance to n+1. The ratios appear to decrease linearly from the nucleus to the edge.

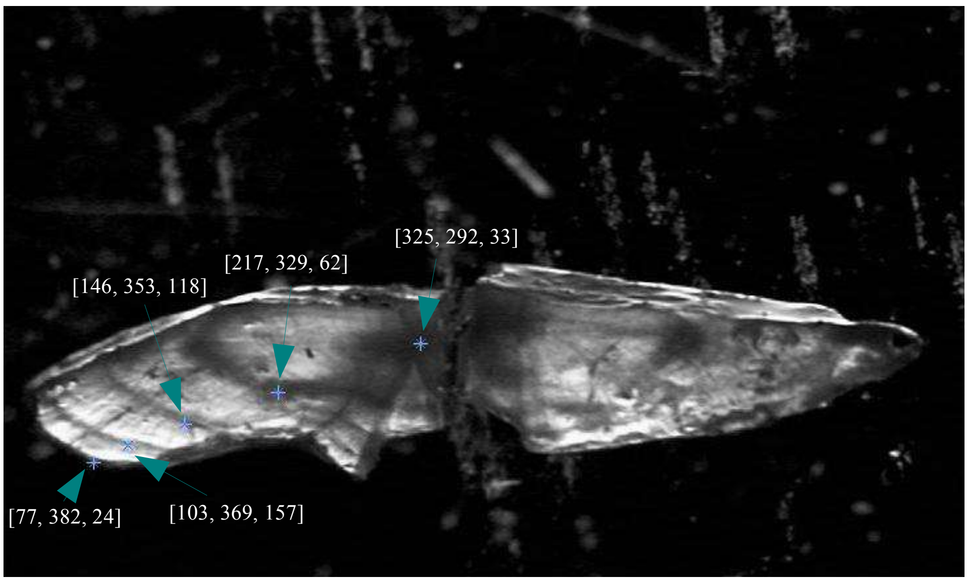


Figure 8.
For this image the manual method was used to count the number of annuli. The user simply clicked the location of the nucleus, each annulus and the edge. At each point the x coordinate, y coordinate, and the intensity value are calculated.

Location on Oto.	***X coordinate***	***Y coordinate***	***Intensity Value***
Nucleus	325	292	33
1st annulus	217	329	62
2nd annulus	146	353	118
3rd annulus	103	369	157
Edge	77	382	24

Table 2.
These are the results of the manual selection from figure 8. For every point selected on the image the program provides the x coordinate, y coordinate, and the intensity value.

	1st annulus	***2nd annulus***	***3rd annulus***	***Edge***
Dis. From Nuc.	114	189	235	264
Ratio(n/n+1)	1.66	1.24	1.12	

Table 3.
The ratios from the manual method are calculated by dividing the distance to the n+1 point by the nth point. Notice as the distance from the nucleus increases the ratios decrease.

3.3

Semi-automatic Count

Using the same image as manual count (largemouth bass age 3). The results from the semi-automatic aging measured three annuli (figure 9). The settings for this measurement had a stringency factor of 0.3 and a 21 by 21 block for the image histogram equalization. The distances found from this trial run were 105 to the first annulus 174 to the second, 229 to the third, and 251 to the edge. The ratios between the annuli are 1.65, 1.32, and 1.09 (table 4). This shows the ratios decreasing as you move away from the nucleus. In this example there were only five rays used to assess the measurement. Each ray estimated 3 annuli providing no standard deviation.

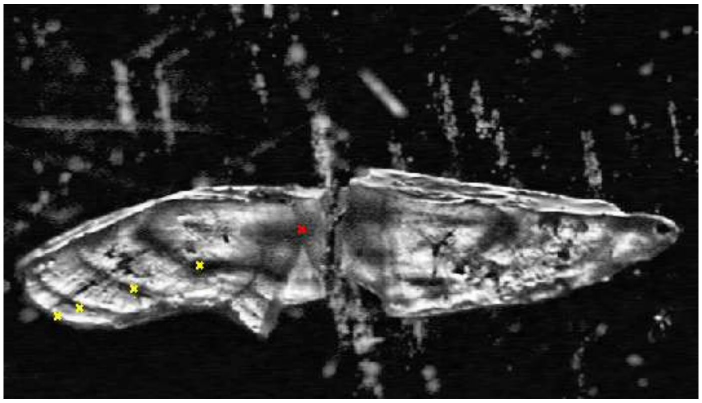


Figure 9.
The results of the semi-automatic count. The yellow x's mark each annulus that was counted as well as the edge. The red x indicated the location of the nucleus.

	1st annulus	***2nd annulus***	***3rd annulus***	***Edge***
Dis. From Nuc.	105	174	229	251
Ratio(n/n+1)	1.65	1.32	1.09	

Table 4.
The ratios from the semi-automatic method are calculated by dividing the distance to the n+1 point by the nth point. Notice as the distance from the nucleus increases the ratios decrease.

3.4

Comparing the two approaches

Comparing the manual to the semi-automatic estimates there is not a significant difference in the ratios (table 5 and figure 10). Both trials seem to have linear relationships. The ratio between the first and second annuli are virtually the same for both methods. This similarity validites the accuracy of the semi-automatic approach.

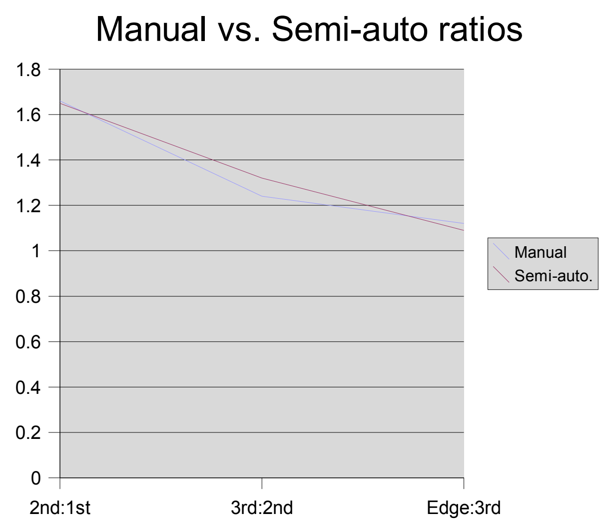


Figure 10.
Annuli ratios for both the manual and semi-automatic approaches. The blue line represents the ratios for the manual method and the red represents the semi-automatic approach.

	2^{nd}:1^{st}	3^{rd}:2^{nd}	Edge:3rd
Manual	1.66	1.24	1.12
Semi-auto.	1.65	1.32	1.09

Table 5.
Comparison between ratios of the manual and semi-automatic methods.

3.5

Graphical User Interface

This final product is an easy to use graphical interface. Figure 11 shows a screenshot of the interface. The user simply browses for the the image of interest and loads it onto the screen. Once the image appears the user can make adjustments as needed. Each adjustment can be monitored every time the user clicks on the adjust image button. The default is for the program to semi-automate the count process. When the image is adjusted correctly the user can then select the manual count radio button to manually measure the image. If the image looks to have a lot of noise the stringency factor can also be adjusted prior to measuring. When everything is adjusted, the user simply clicks the measure button and then measures a triangular area of interest. The results are displayed showing the distances, number of rays used, final count, and standard deviation.

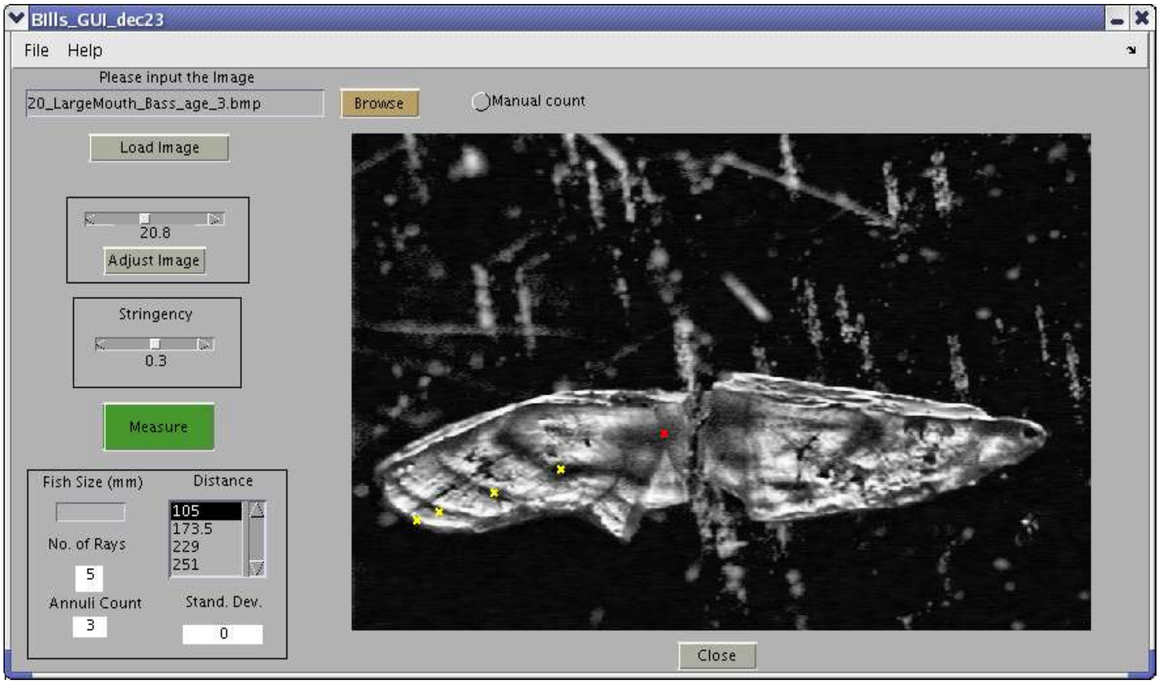


Figure 11.
Snapshot of the graphical user interface.

3.6

Help file

The following is a list of commands that will be available in the user help file:

Parameters

Manual Count	Selecting the manual count button means that the user is choosing the manual approach of counting annuli. Default is set to the semi-automatic approach.
Browse	Pushing this button allows user to look for images by searching files.
Load Image	Once image file is located pushing this button loads the image in the designated area on the right hand side of the graphical user interface.

Adjust image	Pressing this button adjusts and updates the image using an adaptive histogram equilization technique. The range is from two to fifty. The higher the number the more contrasted the image.
Stringency	Adjusting this slider changes how stringent the counting process will be for the semi-automatic approach. The range is from 0.1 ot 0.5. When there is a large amount of image noise, increasing the stringency will help reduce the possiblity of counting false annuli. Default is set to 0.3. The adjustments will not take effect until the meaure button is pressed.
Measure	Once all adjustments are made pressing the measure button will estimate age and determine distance between annuli.
Close	This button closes the program.

Output

Distance	This is the distance from the nucleus to each annulus and the edge. The last number will always be the edge.
Fish size	This box is where the user enters the size of the fish in millimeters. This will later be used for backcalculation.
No. of rays	The number is this box indicates the number of rays used to estimate the annuli count. Used only in the semi-automatic approach.
Annuli count	This box shows the result of annuli estimation.
Stand. Dev.	This box shows the standard deviation of the annuli estimation. For the semi-automatic approach only.

Section 4

Conclusion and Discussion

4.1

Final product

The purpose of this project was to design an easy to use program that would count annuli and measure the annuli ratios. This would help eliminate the headache of having to manually measure distances using an outdated program or even having to physically measure distances under a microscope. With software tools such as Matlab® it is easy to take this one step farther by adding image enhancement properties.

What was initially intended for this program and is actually useful changed during it's creation. Initially this was designed to get an accurate age estimation by analyzing the image. While this program does come up with an estimate it really is no faster than having a reader look at the otolith under a microscope and physically count the annuli. The features that make this program very useful to biologists are the distance ratios. The simplicity of either clicking on the annuli or selecting an area of interest drastically decreases the time needed to find such ratios.

4.2

Problems

Over the course of this project a number of problems occurred. The equipment used to capture the images created much noise surrounding the otolith, which is why image histogram equalization worked so much better than the a regular histogram equalization. As mentioned earlier the image histogram equalization only equalizes in a specified area. If the image capturing equipment was improved perhaps an even more contrasted image would result. With better quality images more of the background noise would be removed. If one could altogether eliminate background noise there are a number of image enhancement techniques that can be applied. For example an edge detection could help isolate the image followed by the use of a series of filters which could leave an image only containing annuli.

The semi-automatic approach by no means is completely accurate especially when there is a lot of noise. An important feature is the yellow and red marks which tell the user where the program calculated either the annuli, the edge, or the nucleus. If the marks are not over any of these features of the otolith it is very simple to remeasure. The marks insure that the estimates are accurate.

Another problem arises with older aged fish. As the number of annuli increases the accuracy of the the program decreases. Part of this is due to the quality of the image. When annuli appear to be closer together it is difficult for the program to distinguish between annuli. That is the reason for the manual count. Again with improved enhancement methods perhaps the semi-automatic approach would increase the accuracy of aging older fish.

4.3

The future

There is still a large amount of work yet to be done with this aging program before it can be used commercially. Currently there is a simple help file that will need to be extended. In addition the creation of an option that allows the user to select a backcalculation model to analyze the fish length:otolith ratios. A database will be established containing the output of the results along with the image. The records could be attached to the file name of the image so that multiple measures of the same image are filed under the same record.

The amount of uses for image analysis in the enviromental field are endless. For example otoliths are also used for the identification of species found in the stomach of marine mammals. Identification based on shape analysis could be yet another project. Beyond otoliths scientists are using geographical information systems to estimate amounts of vegitation by analyzing areal photographs. Jaw images of mammals are used to identify species by meauring suture distances. As technology advances image analysis will begin to play an even larger role in the environmental sciences.

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